

Determination of Menthol in Cigarette Tobacco Filler

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A gas chromatographic and a colorimetric method for determining menthol in cigarette tobacco filler were studied collaboratively by 15 laboratories. No statistically significant differences were found in the precisions within or between laboratories. There were no significant differences between mean values for the two methods for any of the samples. Trends toward higher mean values for the colorimetric method and lower within-laboratory standard deviations for the gas chromatographic method were noted. Remarks by collaborators suggest that the accuracy and precision of the colorimetric method may be improved, and further studies are recommended.

Colorimetric and gas chromatographic procedures have been reported for the determination of menthol in cigarette tobacco (1-3). A collaborative study was conducted on the determination of menthol in cigarette tobacco filler by both a colorimetric and a gas chromatographic method. The Analytical Methods Committee of the Tobacco Chemists' Research Conference chose these methods from those now in use by tobacco companies and related industries engaged in the analysis of menthol in cigarette tobacco.

Three lots of cigarette tobacco were mentholated to about 0.15, 0.25, and 0.33% menthol by spraying with an alcoholic solution of menthol. After thorough mixing, each lot was stored in a large closed container for 6 weeks to facilitate the uniform distribution of menthol throughout the sample. Two completely filled small containers of each sample and one container of nonmentholated control tobacco were sent to each collaborator with a copy of the two methods. Each collaborator was asked to make quadruplicate determinations on each sample by both procedures. Collaborators were asked to report all results to three significant figures although only two figures are usually reported.

METHODS

Colorimetric Method

Apparatus and Reagents

- Distillation setup.*—See Fig. 1.
- Colorimeter.*—Capable of measuring absorbance at 550 m μ .
- Standard menthol solution.*—1 mg/ml. Accurately weigh 100 mg USP 1-menthol into 100 ml volumetric flask, add ethanol to dissolve, and dilute to volume with ethanol.
- Color reagent.*—Prepare dilute sulfuric acid solution (1.6 volumes acid and 1.0 volume water). Dissolve 0.5 g *p*-dimethylaminobenzaldehyde (Eastman, white label) in 100 ml of this dilute acid.
- Ethanol-water mixture.*—Mix equal parts of water and 95% ethanol.

Preparation of Calibration Curve

From standard menthol solution (c), prepare dilute standards by pipetting quantities containing 3, 4, 6, 8, and 10 mg menthol into 100 ml volumetric flasks and diluting to volume with ethanol-water mixture (e).

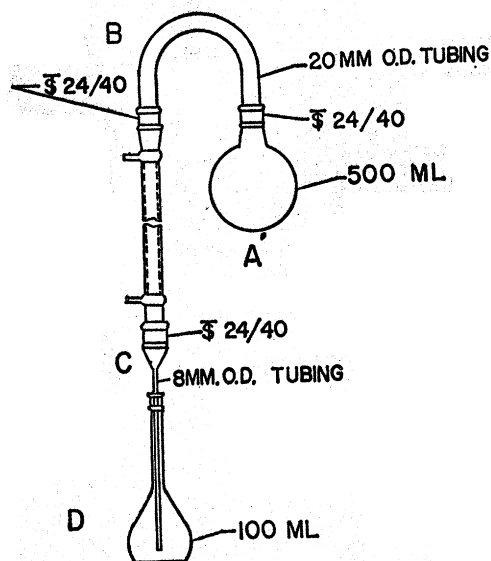


Fig. 1—Distillation setup for colorimetric method. See Determination (Colorimetric Method) for identity of symbols.

Pipet 1 ml of each dilute standard into 10 ml test tube, add 5 ml color reagent, mix, and place in boiling water bath for *exactly* 2 min. Cool in tap water, and within 15 min determine absorbance at 550 m μ , using reagent blank for zero absorbance setting of colorimeter. For reagent blank, add 5 ml color reagent to 1 ml ethanol-water mixture and treat in same manner as standards.

Prepare calibration curve by plotting absorbance vs menthol concentration (mg/100 ml).

Determination

Accurately weigh 2.00–2.15 g cigarette filler and transfer to distillation flask A. Add 80 ml water and a few boiling stones, connect flask A to condenser with tube B, attach adapter C to condenser, and immerse tip in 20 ml ethanol contained in receiving flask.

Gently heat sample flask with Bunsen flame until distillate begins to come over, at which time heat may be increased and receiving flask lowered so that tip of adapter is no longer immersed. Distill until 20 ml distillate have been collected. Disconnect condenser from tube B and wash down condenser with ethanol; remove receiving flask, dilute distillate to ca 70 ml with ethanol, and add water almost to volume. Mix, add ethanol to volume, and mix again.

Pipet 1 ml distillate into 10 ml test tube, add 5 ml reagent, mix, and place in boiling water bath for *exactly* 2 min. Cool in tap water, and within 15 min determine absorbance at 550 m μ , using reagent blank for zero absorbance setting of colorimeter. For the reagent blank, treat 1 ml ethanol-water mixture similarly.

Determine menthol content from calibration curve, and calculate per cent menthol by following equation:

$$\% \text{ Menthol} = (\text{mg menthol/g sample wt}) \times 10.$$

Gas Chromatographic Method

Apparatus and Reagents

(a) *Chromatograph and Column Parameters.*—Equipped with flame ionization detector and controlled injection port and column oven temperature. Column is 5' \times 1/8" stainless steel containing 10% (w/w) silicone oil DC-550 on Chromosorb W, 60/80 mesh. Detector and column are operated at 150°C; nitrogen flow through column is ca 35 ml/min. Injection port temperature is 175°C. Adjust hydrogen and air flows to yield as high a sensitivity as possible and still maintain reasonable stability.

(b) *Mechanical shaker.*—Wrist action.

(c) *Standard menthol-anethole solution.*—0.250 mg menthol and 0.50 mg anethole/ml. Weigh exactly 0.5000 g technical grade anethole into 1 L volumetric flask; wash into flask with several hundred ml 95% ethanol. Weigh exactly 0.2500 g USP 1-menthol and transfer to volumetric flask with enough 95% ethanol to bring to volume. Store solution in dark in glass-stoppered bottle; do *not* use after 6 weeks.

(d) *Extraction solution.*—0.50 mg anethole/ml in ethanol. Weigh exactly 1.000 g anethole into 2 L volumetric flask, make to volume with 95% ethanol, and store in dark.

Preparation of Standard Menthol-Anethole Ratio Factor

Weigh ca 3 g nonmentholated control filler, containing all the usual humectants but no menthol or anethole, into 125 ml flask fitted with rubber stopper. Pipet 50 ml standard menthol-anethole solution (c) into flask, stopper, and shake 1 hr on mechanical shaker. Let solids settle and chromatograph 2 μ l aliquot of supernatant. Repeat twice more to obtain total of 3 replicates of the standard chromatogram. For quantitative results, same person should inject both standard and unknown samples by inserting 2" needle to hilt, injecting 2 μ l rapidly, and withdrawing needle at once. (Menthol elutes in ca 3 min, anethole in ca 5 min.) After ca 10 min all other compounds are eluted, and a new injection can be made.

Draw baselines under menthol and anethole peaks and measure peak heights in mm. Using mean peak height of menthol and anethole from 3 standard chromatograms, calculate standard ratio factor of menthol to anethole by following formula:

Standard ratio factor = peak height for menthol (0.25 mg/ml)/peak height for anethole (0.50 mg/ml).

Determination

Accurately weigh 8–8.5 mentholated cigarette filler and place in 250 ml Erlenmeyer flask fitted with rubber stopper. Pipet 100 ml anethole-ethanol extraction solution (d) into flask, stopper, and shake 1 hr on mechanical shaker. Let sample stand overnight.¹ Chromatograph 2 μ l aliquot of supernatant. Draw

¹ Two hours of continuous shaking will give quantitative results, but for uniformity in this study, follow the above procedure.

baselines under menthol and anethole peaks and measure peak heights in mm. Calculate ratio factor of unknown menthol by following formula:

Ratio factor for unknown = peak height for unknown menthol/peak height for anethole (0.50 mg/ml).

Calculate per cent menthol by following formula:

% Menthol = (unknown ratio factor \times 0.25 \times 10)/(std ratio factor \times g sample wt).

Results and Discussion

Table 1 lists the means of quadruplicate values reported by each collaborator for each method. There are no statistically significant differences in the precisions within or between laboratories. Also, there are no significant differences between means for the two methods for any sample. Two trends are indicated: (a) higher mean values by the colorimetric method, and (b) lower within-laboratory standard deviations by the gas chromatographic method.

The coefficients of variation are lowest with the higher level menthol samples by both methods. Only in the low level sample, which contained less menthol than usually

found in mentholated cigarettes, did the coefficients of variation exceed 10%. Considering the volatility of menthol and the nature of the sample, these values are considered reasonable and within the tolerances expected in processing procedures for the manufacture of mentholated cigarettes.

The mean values obtained by the gas chromatographic procedure are remarkably close to the intended application rates of 0.15, 0.25, and 0.33% menthol.

Collaborators 2 and 8 reported poor baseline separation between the ethanol and menthol peaks in the gas chromatographic method. Collaborator 8 subsequently reported that this difficulty was due to inadequate column conditioning. Collaborators 5, 13, and 14 found that some slight changes in the prescribed column temperature and flow rates were necessary to obtain the expected retention times for menthol and anethole.

Collaborator 8, who routinely uses a similar colorimetric procedure, comments that better precision can be obtained with this method if the samples receive an additional shaking in the bath after 1 minute of

Table 1. Collaborative results for menthol in cigarette tobacco filler by colorimetric (color.) and gas chromatographic (GC) methods

Coll.	Sample 1		Sample 2		Sample 3	
	GC	Color.	GC	Color.	GC	Color.
1	0.243	0.250	0.308	0.330	0.149	0.150
2	0.276	0.276	0.371	0.344	0.180	0.178
3	0.252	0.240	0.340	0.313	0.149	0.145
4	0.228	0.267	0.317	0.334	0.131	0.169
5	0.267	0.247	0.339	0.322	0.164	0.155
6	0.247	0.258	0.326	0.336	0.129	0.129
7	0.269	0.217	0.354	0.333	0.181	0.189
8	0.248	0.257	0.324	0.320	0.156	0.155
9	0.226	0.244	0.304	0.310	0.136	0.152
10	0.254	0.274	0.325	0.317	0.152	0.157
11	0.258	0.304	0.324	0.376	0.156	0.193
12	0.246	0.261	0.309	0.332	0.149	0.183
13	0.243	0.267	0.314	0.332	0.143	0.168
14	0.237	0.274	0.292	0.350	0.126	0.164
15	—	0.267	—	0.329	—	0.159
Mean	0.250	0.260	0.325	0.332	0.150	0.163
Std dev.	0.018	0.019	0.021	0.016	0.017	0.017
Coeff. of var., %	7.2	7.3	6.5	4.8	11.3	10.4
Comparison of Intralaboratory Data						
Av. std dev. ^a	0.0067	0.0108	0.0070	0.0129	0.0060	0.0075

^a Estimated individual laboratory std dev. for each sample calculated from within-laboratory ranges (range \times 0.485).

heating. A colorimetric determination was made on a nonmentholated control by Collaborator 6, who reported a blank value equivalent to a trace of menthol.

Other collaborators reported no real difficulties with either method.

Remarks on the colorimetric method by collaborators suggest that the accuracy and precision of this method may be improved by correcting for a blank value, mixing the sample during color development, and specifying that the cells used to measure absorbance be matched to $\pm 1\%$. Studies on these points are planned.

Recommendation

It is recommended that the study of methods for menthol in cigarette tobacco filler be continued.

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REFERENCES

- (1) Lubus, J. J., and Bogue, H. D., *Tobacco Sci.* 4, 213-215 (1960).
- (2) Mitchell, Betty C., Barbee, E. P., and Irby, R. M., Jr., *ibid.* 7, 64-66 (1963).
- (3) Carpenter, R. D., and Seligman, R. B., Abstracts of Papers, Tenth Tobacco Chemists' Research Conference, Washington, D.C., Nov. 8, 1956.

The recommendation of the Associate Referee was approved by the General Referee and Subcommittee A and was accepted by the Association. See *This Journal* 50, 126 (1967).

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